Stability and cytocompatibility of silk fibroin-capped gold nanoparticles

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ABSTRACT

Surface engineering is crucial in the colloidal stability and biocompatibility of nanoparticles (NPs). Protein silk fibroin (SF), which gained interest in biomaterial and regenerative medicine, was used in this study to stabilize gold (Au) NPs. Characterization results from UV–Vis spectroscopy revealed that SF-capped Au NPs (SF-Au NPs) possessed remarkable colloidal stabilities in the pH range of 2 to 11 and salt concentration range of 50 mM to 1000 mM. In addition, dried particle samples were resuspended after lyophilization without aggregation. The results indicated that the steric hindrance rather than the electrostatic repulsion of SF-Au NPs was essential for colloidal stability. The SF-Au NPs manifested improved cytocompatibility compared with bare Au NPs, which was attributed to the inherent non-cytotoxicity of SF and the good colloidal stability of the NPs. The proposed method was simpler, more efficient, and more cost effective than the conventional modification strategies for Au NPs; thus, SF-Au NPs can be potentially used in biomedical applications.

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1. Introduction

Gold nanoparticles (Au NPs) have gained considerable interest for potential applications in biological and medical fields, such as biosensors, drug delivery, and diagnostic assays, because of their unique physical and chemical properties [1–3]. The simplest and most commonly used preparation route for Au NPs is the aqueous reduction of Au salt by sodium citrate under reflux [4]. However, the prepared Au NPs are susceptible to aggregation at high ionic strengths or acidic conditions because of the changes in particle surface charge [5]. Although the aggregation of Au NPs is beneficial for certain events of bimolecular recognition, the particles should be resistant to aggregation caused by variation in pH or environments with high ionic strengths. The toxicity of Au NPs from a biological perspective is likewise critical [6].

Most biomedical applications recommend water-soluble Au NPs that exhibit stability and biocompatibility [7]. Modifying the surface of NPs with specific ligands is an effective approach for protecting against unselective particular aggregation. Previous studies have evaluated various surface ligands, such as surfactants [8], thiol compounds [7,9–11], dendrimers [12–14], polymers [15,16], and zwitterionic materials [17–19]. However, most of these ligands suffer from poor biocompatibility, requirement of multiple synthetic steps, or expensive reagents.

Silk fibroin (SF) is secreted from silk glands of the silkworm *Bombyx mori*. SF proteins belong to a class of unique, block copolymer-like proteins with high molecular weights [20]. Raw and regenerated SF has been extensively used in biomaterials and regenerative medicine

because of its biocompatibility and nontoxicity [21–23]. The incorporation of SF proteins with colloidal Au NPs might have potential bioactivities for biotechnological applications.

In the present study, we used SF proteins to stabilize Au NPs (Scheme 1). The colloidal stabilities of SF-capped Au NPs (SF-Au NPs) were tested under different pH values and salt concentrations by UV–Vis spectroscopy. The resuspension property of dried samples after freeze-drying was also evaluated. The cytotoxicity of SF-Au NPs was analyzed by standard MTT assay. Our method was simpler and more cost effective compared with the current methods for the modification and conjugation of Au NPs; thus, our method was promising for various biomedical applications.

2. Experimental

2.1. Materials

$HAuCl_4 \cdot 3H_2O$ was purchased from Beijing Chemical Co. (Beijing, China). Bovine serum albumin (BSA) was bought from Shanghai Sangon (Shanghai, China). Simulated body fluid (SBF; 1.5-fold) was prepared as previously described [24]; pH was adjusted to 7.4 with HCl. All other reagents were of analytical grade and used as received. All aqueous solutions were prepared with triple distilled water.

2.2. Preparation of SF-Au NPs

Scheme 1 illustrates the preparation of aqueous SF solutions based on a previous report [25]. The SF solution was dialyzed against distilled water using Slide-a-Lyzer dialysis cassettes (MWCO 3500, Pierce) for 3 d (triple-distilled water for the last day) until the

conductivity of the dialyzed water was close to that of distilled water. The molecular weight of the as-prepared SF was measured to be approximately 25 kDa to 86 kDa via SDS-PAGE (Fig. S1).

The absorbance peak at 275 nm (A_{275}) did not significantly change within pH 3 to 9 (Fig. S2). A_{275} gradually increased with the weight concentration of SF; a good linearity was observed between A_{275} and SF concentration below 2 $mg \cdot mL^{-1}$ (Fig. S3). Thus, the weight concentration of SF can be obtained from A_{275}.

Au NPs were synthesized by citrate reduction of $HAuCl_4$ as previously described [26]. The diameter of the NPs based on TEM images was 13 ± 2 nm. The Au NP concentration (ca. 15 nM) was determined from Beer's law using an extinction coefficient of ca. 10^8 $M^{-1} \cdot cm^{-1}$ at 520 nm [27].

SF-Au NPs were obtained by mixing 300 μL of as-prepared Au NP solution with SF solution at ambient temperature, followed by incubation for 10 min, the final volume was 1 mL and the pH of the mixture was about 7. The final concentrations of SF and Au NPs were 2 $mg \cdot mL^{-1}$ and 4.5 nM, respectively.

2.3. Colloidal stabilities of SF-Au NPs to pH and salts

To determine the stability of the Au NPs with respect to pH, the solutions of SF-Au NPs and Au NPs were added to 20 mM tris(hydroxymethyl)aminomethane–2-(N-morpholino)ethanesulfonic acid (Tris–MES) or 20 mM phosphate buffer (PB) at pH 2 to 11 (volume ratio, 1:1). The stability of the Au NPs with respect to various salt concentrations was determined by adding 20 mM PB solution at pH 7.4 to the solutions of SF-Au NPs and Au NPs; the PB contained twofold of the desired salt concentrations (volume ratio, 1:1). The stability of the Au NPs in the physical environment was probed by adding 1.5-fold SBF solutions (volume ratio, 1:2) to the solutions of SF-Au NPs and Au NPs. The final concentrations of SF and Au NPs in all the solutions were 2 $mg \cdot mL^{-1}$ and 4.5 nM, respectively. The stabilities of the SF-Au NPs were investigated via UV–Vis spectroscopy.

2.4. Cytotoxicity assay and cell morphology observation

Human umbilical vein endothelial cells (HUVECs) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 $U \cdot mL^{-1}$ penicillin, and 100 $mg \cdot mL^{-1}$ streptomycin; the HUVECs were then cultured at 37 °C in 5% CO_2 humidified environment.

Cytotoxicity was assessed by standard MTT assay. The HUVECs were plated at a density of 5×10^3 cells/well in a 96-well plate and then cultured for 24 h. The medium was replaced with fresh medium containing different concentrations of the cit-Au NPs and SF-Au NPs; the Au atomic concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS). The cells cultured in NP-free media were used as controls. The wells were washed with PBS and the medium was replaced with 100 mL of fresh medium after 24 h of treatment. Up to 20 mL of MTT (5 $mg \cdot mL^{-1}$) was added to each well;

the cells were further cultured at 37 °C for 4 h. Dark blue formazan crystals generated by mitochondria dehydrogenase in live cells were dissolved in 150 mL of dimethyl sulfoxide (DMSO); the absorbance at 570 nm was measured by a microplate reader (MODEL 550, Bio Rad, USA). The relative cell viability is given as

Relative cell viability (%)
= absorption of treated well/absorption of control well × 100. (1)

Five replicates were obtained for each sample, and the mean value was used as the final result. All data were presented as mean ± SD. Statistical analysis of data was made by ANOVA or Student's t-test. It was considered significantly different if $p < 0.05$.

The working solution was freshly prepared by adding 5 μL of fluorescein diacetate (FDA, 5 $mg \cdot mL^{-1}$ acetone) stock solution to 5 mL of PBS. Up to 20 μL of FDA working solution was added to each well and then incubated for 15 min. The wells were washed twice with PBS; confocal laser scanning microscopy (CLSM, BIORAD 2000) was carried out to observe the cell morphology.

2.5. Characterization

UV–Vis spectra were obtained with a UV/Vis Shimadzu UV-2505 spectrometer. TEM was performed on a JEM-1200EX (JEOL, Japan). Samples for TEM measurements were prepared by placing a drop of colloidal dispersion on a carbon-coated copper grid, followed by evaporation of the solvent. Samples were freeze-dried completely before being grinded into powder and pressed into pellets with KBr for measuring infrared spectra with a Fourier transform infrared spectrophotometer (FTIR) (Bruker VERTEX80 V, Germany).

3. Results and discussion

3.1. Characterization of citrate-capped Au NPs (cit-Au NPs) and SF-Au NPs

A characteristic absorption peak at 520 nm was observed in the UV–Vis spectra of cit-Au NPs (diameter, ~13 nm). As shown in Fig. 1a, the aqueous solution of SF-Au NPs displayed another strong absorption band at approximately 275 nm after the addition of 2 $mg \cdot mL^{-1}$ SF to the solution of cit-Au NPs. This band was assigned to the $\pi \rightarrow \pi^*$ electron transition of the tyrosine (Tyr) residue in the SF molecular chain. A 5 nm red shift was observed from 520 nm to 525 nm, and the peak absorbance of the Au NPs increased, indicating the adsorption of SF onto the surface of the Au NPs [28]. No statistical differences in the average particle diameter or size distribution of the Au NPs were detected from the TEM images (Figs. 1b and c). The SF-Au NPs remained well separated, which implied the absence of aggregation.
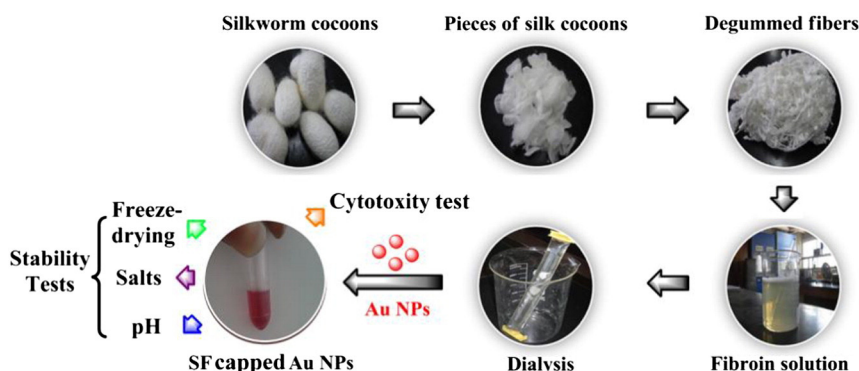
Scheme 1. Preparation and stability tests of SF capped Au NPs.